REMARKS

In the Claims

New claim 25 has been added. Support for claim 25 is found, for example, on page 5, lines 11-14, of the specification as filed.

Rejection of Claims 9-16 Under 35 U.S.C. §103(a)

The Examiner has rejected claims 9-16 under 35 U.S.C. §103 as being obvious over WO 98/25637 and U.S. Patent No. 6,793,939 each in view of Saad et al. (*In Vitro Cell Dev Biol*, 1993, 29A:32-40; hereinafter "Saad et al."). WO 98/25637 and U.S. Patent No. 6,793,939 are the PCT publication and the issued U.S. patent, respectively, based on the PCT application from which the WO 98/25637 publication resulted. WO 98/25637 and U.S. Patent No. 6,793,939 are collectively referred to hereinafter as "Badylak."

The Examiner contends that Badylak teaches methods of inducing endogenous tissue formation at a site in need thereof by administering a graft composition comprising liver basement membrane in an amount effective to induce the repair of the tissue at the site of administration. The Examiner states that Badylak further describes that eukaryotic cells may be seeded onto the liver basement membrane prior to implantation. However, according to the Examiner, Badylak does not disclose administering a graft comprising liver basement membrane with hepatocytes cultured thereon in order to repair damaged or diseased liver tissue. Furthermore, the Examiner concedes that Badylak does not teach the functionality of the hepatocytes once cultured on the liver basement membrane.

The Examiner asserts that Saad et al. teaches that crude liver membrane fractions obtained from the liver support growth of cultured hepatocytes and allow the

hepatocytes to retain liver specific activities (e.g., the production of albumin and cytochrome P450 enzymes). Furthermore, the Examiner argues that Saad et al. suggests the natural mix of membrane proteins and extracellular signaling molecules available in the crude membrane fractions are responsible for supporting the liver specific functions of the hepatocytes.

The Examiner contends that it would have been within the knowledge of one skilled in the art to employ the hepatocyte culturing technique of Saad et al. with the liver basement membrane used by Badylak to arrive at a method where hepatocytes and stromal cells may be co-cultured on liver basement membrane for administration to a patient in need of liver repair. The Examiner further contends that one would have had a reasonable expectation that the liver basement membrane would have successfully supported hepatocyte growth so that the hepatocytes are at least capable of maintaining their function *in vitro*, and, thus, have an effect upon *in vivo* administration. Applicants respectfully traverse the Examiner's rejection. Claims 9-16 of the instant application are not obvious over Badylak in view of Saad et al.

The mere fact that references can be combined or modified does not render the resultant combination obvious unless the results would have been predictable to one of ordinary skill in the art. *KSR International Co. v. Teleflex Inc.*, 82 USPQ2d 1385, 1396 (2007). Although Applicants disagree that the Examiner has established a *prima facie* case of obviousness sufficient to support the Examiner's rejections under 35 U.S.C. §103, the Examiner's rejections under 35 U.S.C. § 103(a) are overcome for the following reasons.

A. REFERENCES MUST BE CONSIDERED AS A WHOLE, INCLUDING PORTIONS THAT WOULD LEAD AWAY FROM THE CLAIMED INVENTION

The Examiner asserts that Saad et al. establishes that means were known in the art, before the filing date of the instant application, for culturing hepatocytes while

maintaining their functionality. However, Saad et al. teaches away from claims 9-16 of the instant application. Ascertaining the differences between the prior art and the claims at issue requires interpreting the claim language, and considering both the invention and the prior art references as a whole. A prior art reference must be considered in its entirety, i.e., as a whole, including portions that would lead away from the claimed invention. *W.L. Gore & Associates, Inc. v. Garlock, Inc.*, 721 F.2d 1540, 220 USPQ 303 (Fed. Cir. 1983), *cert. denied*, 469 U.S. 851 (1984) and MPEP § 2145.

Independent claim 9 specifies that the "graft composition is prepared by providing liver basement membrane substantially free of cells and seeding the hepatocytes on the liver basement membrane substantially free of cells." Saad et al. teaches the preparation of crude liver membrane fractions from liver cells for use as a substrate for the *in vitro* culture of rat hepatocytes. As described in Saad et al., the crude liver membrane fractions are obtained by three steps of hypotonic lysis and homogenization of liver cells with each step followed by low-speed centrifugation to remove particulate material not susceptible to disruption by hypotonic lysis and homogenization (see page 33, column 1, paragraph entitled "Preparation of Crude Membrane Fractions"). The supernatants are collected following each centrifugation step and the membranes are isolated from the supernatant fractions. The supernatants contain the crude liver cell membranes and these crude liver cell membrane fractions constitute a ***cellular membrane fraction*** from liver cells.

First, Saad et al. teaches away from using an extracellular matrix fraction to form a graft comprising hepatocytes. Saad et al. describes that "[e]lectron micrographs of [crude membrane fractions] showed a ***homogenous plasma membrane fraction***, with only occasional contaminations by lipid droplets or mitochondria." (see Saad et al., page 34, first column, first full paragraph) (emphasis added). This ***plasma membrane fraction*** is a ***cellular***

membrane fraction. Moreover, because the plasma membrane fraction is described as ***homogenous***, the culture substrate used in Saad et al. is comprised only of ***cellular membranes***.

In contrast, the invention as defined by claims 9-16 of the instant application is completely counter to the method described in Saad et al. Claim 9 describes the maintenance of functional hepatocytes by seeding the hepatocytes on *liver basement membrane substantially free of cells*. The liver basement membrane described in claim 9 is comprised of ***extracellular matrix*** components, in contrast to the ***cellular membrane fraction*** described in Saad et al.

In fact, Saad et al. teaches away from using an extracellular matrix fraction for maintaining functionality of hepatocytes. Specifically, Saad et al. teaches that

> "[A]lthough hepatocytes attached efficiently to [collagen] and remained viable, such cultures were unable to maintain the expression of many liver-specific functions over prolonged culture time, such as enzymes involved in xenobiotic metabolism. *Similar problems have been encountered in cultures on components from extracellular matrix and membranes*, including type IV collagen, laminin, and fibronectin."

(see Saad et al., page 36, second column, second full paragraph) (emphasis added). Thus, Saad et al. teaches that an extracellular matrix construct is problematic for maintaining the functionality of hepatocytes. Consequently, Saad et al. teaches away from Applicants' claimed invention wherein extracellular matrix composition is utilized to maintain hepatocyte functionality.

Second, Saad et al. teaches that the crude liver cell membrane fractions used to grow hepatocytes "included membranes from all liver cells, from nonparenchymal and parenchymal cells." (See Saad et al., page 37, second column). Moreover, Saad et al. teaches that "[c]ell-to-cell contact between hepatocytes and nonparenchymal rat liver cells, but not gap-junctions or soluble factors, has been shown to be ***essential*** for the expression of liver-specific functions in long-term cultures." *Id.* (emphasis added). Accordingly, Saad et al. teaches that the presence of liver cell components (i.e., nonparenchymal liver cells) in the crude liver membrane fractions used for culturing hepatocytes are ***essential*** to the liver-specific functions of hepatocytes.

Thus, Badylak, as indicated by the Examiner, does not report on the functionality of hepatocytes cultured on liver basement membrane (see page 6 of the October 9, 2009 Office Action). Further, Saad et al. teaches away from Applicants' claimed invention, i.e., the maintenance of ***functional*** hepatocytes by seeding the hepatocytes on ***liver basement membrane substantially free of cells***, because 1) Saad et al. teaches that cultures comprising extracellular matrix components are problematic for maintaining the expression of liver-specific functions in hepatocyte culture and 2) Saad et al. teaches that liver cell components in the crude liver membrane fractions (i.e., nonparenchymal liver cells) are ***essential*** for cultured hepatocytes to retain liver-specific functions. Therefore, the reference combination does nothing to render claims 9-16 of the instant application obvious. In fact, Saad et al. teaches away from the claimed invention. Accordingly, withdrawal of the rejection of claims 9-16 under 35 U.S.C. §103(a) is respectfully requested.

B. GREATER THAN EXPECTED RESULTS ARE EVIDENCE OF NONOBVIOUSNESS-

Applicants have shown that ***functional*** hepatocytes can be maintained by seeding the hepatocytes on ***liver basement membrane substantially free of cells***. As the

court concluded in *In re Diamond*, the question of nonobviousness must turn on whether the *prima facie* case of obviousness of the claimed composition is rebutted by a showing of unexpected results. *In re Diamond*, 53 CCPA 1172, 360 F.2d 214, 149 USPQ 562 (1966). See also *In re Meinhardt*, 55 CCPA 1000, 392 F.2d 273, 157 USPQ 270 (1968). A showing that the results obtained were greater than those which would have been expected from the prior art, and that the results are of a significant, practical advantage, is evidence of nonobviousness. *See* MPEP § 7.16(a); *Ex parte The NutraSweet Co.*, 19 USPQ2d 1586 (Bd. Pat. App. & Inter. 1991).

As described above, Saad et al. describes the use of a ***plasma membrane fraction*** that is comprised only of ***cellular membranes*** as a culture substrate. Moreover, Saad et al. teaches that an extracellular matrix construct is problematic for maintaining the functionality of hepatocytes. In contrast, Applicants' claim 9 describes the maintenance of functional hepatocytes by seeding the hepatocytes on ***liver basement membrane substantially free of cells***. Liver basement membrane is comprised of ***extracellular matrix*** components, which is inapposite to the ***cellular membrane fraction*** described in Saad et al.

Furthermore, Saad et al. describes growth of hepatocytes on crude liver membrane fractions, which include "membranes from all liver cells, from nonparenchymal and parenchymal cells." (See Saad et al., page 37, second column). Saad et al. suggests that cell-to-cell contact between hepatocytes and nonparenchymal liver cell components are required for the expression of liver-specific functions. *Id.* Therefore, one skilled in the art would not expect to maintain ***functional*** hepatocytes by seeding the hepatocytes on ***liver basement membrane substantially free of cells***, as specified in Applicants' claims 9-16.

Specifically, hepatocyte functionality on liver basement membrane was found by Applicants to be unexpectedly superior to conventional hepatocyte culture on adsorbed

collagen and hepatocytes were found to maintain synthetic and metabolic functions when cultured on liver basement membranes substantially free of cells. For example, albumin production, a measure of liver synthetic function, was found to be maintained or elevated in hepatocytes cultured on substantially cell free liver basement membrane, whereas albumin production declined in hepatocytes cultured on adsorbed collagen. (See page 22, lines 19-21 in the instant application). The synthetic and metabolic functions were not only superior to culture on adsorbed collagen, but were also comparable to culture on a double-gel collagen substrate, a substrate with known capacity for maintaining hepatocyte synthetic and metabolic functions. (See page 22, lines 16-18 in the instant application).

Also, urea content, a measure of liver metabolic function, was found to be about the same in hepatocytes cultured on liver basement membrane substantially free of cells, on a per cell basis, as that from cells grown on a double-gel substrate. (See page 22, line 32 to page 23, line 1 in the instant application). Additionally, in hepatocytes cultured on liver basement membrane substantially free of cells, cytochrome P450 activity, a measure of liver metabolic activity, was at least as high if not greater than that for hepatocytes grown on a double-gel substrate. (See page 23, lines 27-31 in the instant application). These results together show that hepatocytes grown on liver basement membranes substantially free of cells exhibit specific liver synthetic and metabolic activity characteristic of *functional hepatocytes*, results that are unexpected based on the prior art.

Accordingly, even if the Examiner has established a *prima facie* case of obviousness, and Applicants contend that the Examiner has not, Applicants have overcome the Examiner's *prima facie* case of obviousness by demonstrating that Applicants' claimed methods and compositions unexpectedly result in a level of hepatocyte functionality that is difficult to obtain. Furthermore, as discussed above, Saad et al. strongly teaches away from

Applicants' claimed invention, and the cited reference combination does nothing to render claims 9-16 of the instant application obvious. Withdrawal of the rejection of claims 9-16 under 35 U.S.C. § 103(a) is respectfully requested.

Double Patenting

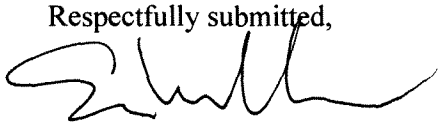
(1) The Examiner has rejected claims 9-16 on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-14 of U.S. Patent No. 6,793,939 in view of WO 98/25637, and further in view of Saad et al. Independent claim 9 specifies that the graft composition is prepared by seeding hepatocytes on liver basement membrane substantially free of cells. Accordingly, claims 9-16 are not obvious over claims 1-14 of U.S. Patent No. 6,793,939 in view of WO 98/25637, and further in view of Saad et al. The claims of U.S. Patent No. 6,793,939 provide no suggestion that ***functional*** hepatocytes can be maintained in culture by seeding the hepatocytes on ***liver basement membrane substantially free of cells***. Furthermore, the secondary references cited by the Examiner do not provide what the claims of U.S. Patent No. 6,793,939 are missing (*see* arguments above for the rejection under 35 U.S.C. § 103). In addition, Saad et al. teaches away from Applicants' claimed invention because 1) Saad et al. teaches that cultures comprising extracellular matrix components are problematic for maintaining the expression of liver-specific functions in hepatocyte culture and 2) Saad et al. teaches that liver cell components in the crude liver membrane fractions (i.e., nonparenchymal liver cells) are ***essential*** for cultured hepatocytes to retain liver-specific functions. Accordingly, claims 9-16 cannot be obvious over claims 1-14 of U.S. Patent No. 6,793,939 in view of WO 98/25637, and further in view of Saad et al. Applicants respectfully request that the rejection of claims 9-16 on the basis of obviousness-type double patenting be withdrawn.

(2) The Examiner has rejected claims 9 and 12 on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1, 3, 12, and 14 of U.S. Patent No. 7,482,025 in view of WO 98/25637, and further in view of Saad et al. Independent claim 9 specifies that the graft composition is prepared by seeding hepatocytes on liver basement membrane substantially free of cells. Accordingly, claims 9 and 12 are not obvious over claims 1, 3, 12, and 14 of U.S. Patent No. 7,482,025 in view of WO 98/25637, and further in view of Saad et al. The claims of U.S. Patent No. 7,482,025 provide no suggestion that ***functional*** hepatocytes can be maintained in culture by seeding the hepatocytes on ***liver basement membrane substantially free of cells***. Furthermore, the secondary references cited by the Examiner do not provide what the claims of U.S. Patent No. 7,482,025 are missing (*see* arguments above for the rejection under 35 U.S.C. § 103). In addition, Saad et al. teaches away from Applicants' claimed invention because 1) Saad et al. teaches that cultures comprising extracellular matrix components are problematic for maintaining the expression of liver-specific functions in hepatocyte culture and 2) Saad et al. teaches that liver cell components in the crude liver membrane fractions (i.e., nonparenchymal liver cells) are ***essential*** for cultured hepatocytes to retain liver-specific functions. Accordingly, claims 9 and 12 cannot be obvious over claims 1, 3, 12, and 14 of U.S. Patent No. 7,482,025 in view of WO 98/25637, and further in view of Saad et al. Applicants respectfully request that the rejection of claims 9 and 12 on the basis of nonstatutory obviousness-type double patenting be withdrawn.

CONCLUSION

The foregoing amendments and remarks are believed to fully respond to the Examiner's rejections. The claims are in condition for allowance. Applicants respectfully request allowance of the claims, and passage of the application to issuance.

Respectfully submitted,



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